Developmental regulation of adipose tissue growth through hyperplasia and hypertrophy in the embryonic Leghorn and broiler

P. R. Chen, Y. Suh, Y. M. Choi, S. Shin, and K. Lee

Department of Animal Sciences, The Ohio State University, Columbus, OH

INTRODUCTION

Minimizing adipose accumulation has surfaced as a new target in the poultry industry to increase production and performance. However, insufficient information has been collected on embryonic adipose tissue growth and its potential influence on post-hatch development. Furthermore, humans and chickens exhibit similar means of adipose growth, which may allow embryonic chickens to be used as models for early mechanisms of obesity.

Hyperplasia, an increase in cell number, and hypertrophy, an increase in cell size, are the two mechanisms by which embryonic adipose tissue grows. As development progresses, there is a dramatic increase in the expression of the adipocyte differentiation marker, fatty acid binding protein 4 (**FABP4**), and TG breakdown is catalyzed by adipose triglyceride lipase (**ATGL**) at the end of incubation.

The aim of this study was to understand adipocyte growth mechanisms of hyperplasia and hypertrophy throughout embryonic development and compare the dynamics of adipose growth between layer-type and meat-type chickens. This will provide fundamental information on the morphology, proliferation, and differentiation of adipocytes and potential similarities or differences during embryonic development of both breeds.

OBJECTIVES

1. To measure DNA contents, which correlates to hyperplasia, within embryonic chicken adipose tissue of layer-types and meat-types.
2. To histologically examine the morphology and size of adipocytes to determine the extent of hypertrophy in both chicken breeds.
3. To determine the expressions of the target differentiation marker and TG breakdown factor by western blot analysis in both breeds.

MATERIALS AND METHODS

1. Leghorn (layer) and broiler (meat) chickens
   - Embryonic day 12, 14, 16, 18, 20 (E12-E20) and day 1 post-hatch (D0).
   - 15 embryos or chicks collected per breed at each time point.
   - Neck and leg fat pads excised and weighed for analyses.
2. Adipose samples of embryos or chicks subjected to:
   - Total DNA extraction (n=5 per breed) → Cell number
   - Tissue sectioning and histology (n=5 per breed) → Cell Size
   - Western blot analysis of FABP4 and ATGL (n=5 per breed) → Differentiation and TG breakdown markers

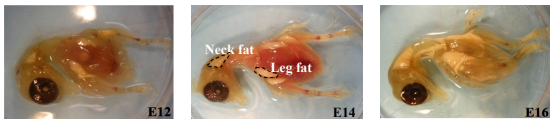


Figure 1. Leghorn fat pad visualization at E12, E14, and E16. Left neck fat pad and right leg fat pad are indicated by dashed lines on E14 embryo. Images not to scale.

RESULTS

Embryo or Body Characteristics

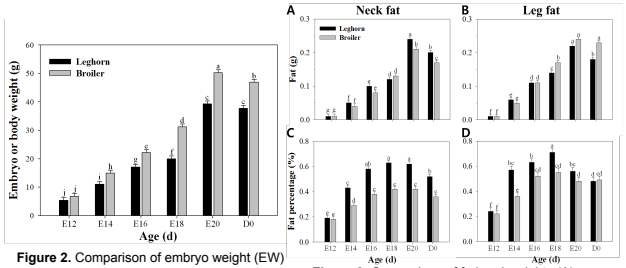


Figure 2. Comparison of embryo weight (EW) from embryonic day 12 (E12) to embryonic day 20 (E20) and body weight (BW) at day 1 post-hatch (D0) between Leghorn and broiler. Bars represent mean ± SEM. Significance (P<0.05) indicated by different letters.

Figure 3. Comparison of fad pad weights (A) in the neck (B) and in the leg (C) with neck fat percentage (D) and leg fat percentage relative to EW or BW between Leghorn and broiler.

- Broiler embryonic weight (EW) or body weight (BW) was significantly higher than Leghorn from E14 onward.
- Notable increase in EW and fat pad weights from E18 to E20.
- Decrease in BW and fat pad weights from E20 to D0.
- Fat pad weight relative to EW or BW significantly lower for broiler.

DNA Contents

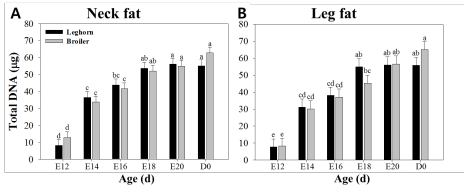


Figure 4. Comparison of total DNA contents (A) within the neck fat pad (B) and the leg fat pad between Leghorn and broiler.

- Significant increase in DNA content from E12 to E14 in both breeds.
- DNA content achieves steady state around E18, mainly in Leghorn.

Morphological Characteristics

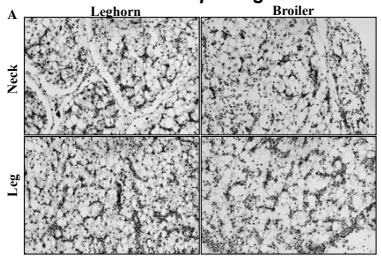
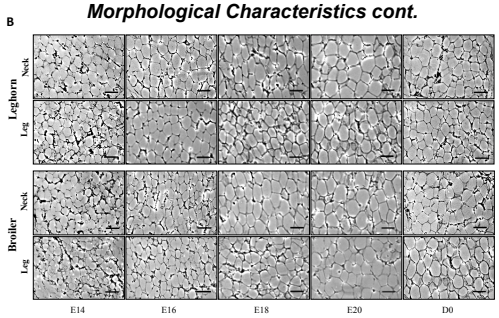


Figure 5. Histological images of (A) embryonic day 12 (E12) neck and leg adipose tissue at 200x (B) and subsequent time points (E14-D0) for Leghorn and broiler at 100x. Scale bar = 50μm.

- Clusters of preadipocytes.
- Multilocular and unilocular adipocytes.

Morphological Characteristics cont.



- Expansion of adipocyte size from E14 to E20 (unilocular).
- Slight decrease in adipocyte size from E20 to D0.
- Leghorn adipocyte cross-sectional area (μm²) tended to be larger than broiler (data not shown).

Western Blot Analysis

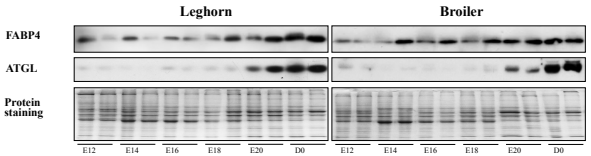


Figure 6. Leghorn and broiler expression levels of FABP4 and ATGL from embryonic day 12 (E12) to day 1 post-hatch (D0). Coomassie blue protein staining was used as a standard.

- FABP4 expression increases with each time point → maturing adipocytes.
- ATGL up-regulated at E20 and D0 → mobilize lipid for hatching.

CONCLUSIONS

1. Preadipocytes differentiate in the middle of embryonic development (E12-E14), and hyperplasia is active during this time.
2. Throughout the second half of embryonic development (E14- E20), adipocytes continually recruit lipid to grow by hypertrophy.
3. Protein expressions (FABP4 and ATGL) are relatively similar between both breeds during embryonic stages.
4. Adipose tissue development is not different between layers and meat-type chicken embryos, suggesting post-hatch events account for differences.

ACKNOWLEDGMENTS

This work was supported by an Ohio Agricultural Research and Development Center SEEDS grant, a Sigma Xi Grant-in-Aid of research, and a grant from the Next-Generation BioGreen 21 Program (No. PJ009457), Rural Development Administration, Republic of Korea.